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Redistribution of Hepatocyte Chloride during L-Alanine Uptake

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Abstract. We used ion-sensitive, double-barrel microelectrodes to measure changes in hepatocyte transmembrane potential (V_m), intracellular K^+, Cl^-, and Na^+ activities (a^i_K, a^i_{Cl} and a^i_{Na}), and water volume during L-alanine uptake. Mouse liver slices were superfused with control and experimental Krebs physiological salt solutions. The experimental solution contained 20 mM L-alanine, and the control solution was adjusted to the same osmolality (305 mOsm) with added sucrose. Hepatocytes also were loaded with 50 mM tetramethylammonium ion (TMA^+) for 10 min. Changes in cell water volume during L-alanine uptake were determined by changes in intracellular, steady-state TMA^+ activity measured with the K^+ electrode. Hepatocyte control V_m was -33 ± 1 mV. L-alanine uptake first depolarized V_m by 2 ± 0.2 mV and then hyperpolarized V_m by 5 mV to -38 ± 1 mV ($n = 16$) over 6 to 13 min. During this hyperpolarization, a^i_{Na} increased by 30% from 19 ± 2 to 25 ± 3 mM ($P < 0.01$), and a^i_K did not change significantly from 83 ± 3 mM. However, with added ouabain (1 mM) L-alanine caused only a 2-mV increase in V_m, but now a^i_K decreased from 61 ± 3 to 54 ± 5 mM ($P < 0.05$). Hyperpolarization of V_m by L-alanine uptake also resulted in a 38% decrease of a^i_{Cl} from 20 ± 2 to 12 ± 3 mM ($P < 0.001$). Changes in V_m and $V_{Cl} - V_m$ voltage traces were parallel during the time of L-alanine hyperpolarization, which is consistent with passive distribution of intracellular Cl^- with the V_m in hepatocytes. Added Ba^{2+} abolished the L-alanine-induced hyperpolarization, and a^i_{Cl} remained unchanged. Hepatocyte water volume during L-alanine uptake increased by $12 \pm 3\%$. This swelling did not account for any changes in ion activities following L-alanine uptake. We conclude that hepatocyte a^i_K is regulated by increased Na^+-K^+ pump activity during L-alanine uptake in spite of cell swelling and increased V_m due to increased K^+ conductance. The hyperpolarization of V_m during L-alanine uptake provides electromotive force to decrease a^i_{Cl}. The latter may contribute to hepatocyte volume regulation during organic solute transport.

Key words: Liver — L-alanine — Chloride — Cell volume regulation — Membrane potential — Ion-sensitive microelectrodes

Introduction

L-Alanine influx into hepatocytes is predominantly Na^+ dependent and is stimulated by intracellular negativity (Kristensen, 1980; 1986). The stoichiometry between cotransported Na^+ and alanine is 1:1 (Kristensen & Folke, 1983). Thus, the transmembrane Na^+ electrochemical gradient seems to be the exclusive driving force for intracellular alanine accumulation. At least eight distinct amino acid transport systems have been identified in hepatocytes. System A accounts for most of the alanine uptake (Kilberg, 1982; Wondergem & Castillo, 1988).

Na^+-coupled alanine influx in hepatocytes is accompanied by changes in other transport processes. The membrane potential undergoes a transient depolarization followed by sustained hyperpolarization (Fitz & Scharschmidt, 1987*b*; Wondergem & Castillo, 1988). An increase in hepatocyte intracellular Na^+ accompanied by a decrease of intracellular K^+ also has been reported (Kristensen, 1986). The lat-

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ter, however, was not confirmed by electron probe microanalysis of K^+ during L-alanine uptake in cultured hepatocytes, in spite of evidence for increased K^+ efflux induced by L-alanine (Cohen & Lechene, 1990). Brisk activation of the Na^+-K^+ pump, resulting from increases in cell Na^+ via the Na-alanine cotransport, apparently compensates alanine-induced loss of cell K^+ (Kristensen, 1980; Cohen & Lechene, 1990). This alanine-induced increase in K^+ permeability and K^+ efflux compensates the cell swelling due to accumulation of L-alanine, and it also accounts for the hyperpolarization of the hepatocyte transmembrane potential, V_m (Kristensen, 1980; Bear & Petersen, 1987; Wondergem & Castillo, 1988; Bear, 1990).

Little is known about the effect of L-alanine transport on hepatocyte Cl^-. Cl^- distributes passively with V_m in hepatocytes (Lyall, Croxton & Armstrong, 1987; Fitz & Scharschmidt, 1987*a*; Wang & Wondergem, 1992), and an inverse correlation between the Cl^- distribution and the initial rate of alanine transport has been shown by Moule and colleagues (1987).

We have demonstrated previously that hepatocyte intracellular Cl^- distributes passively with V_m in both iso- and anisosmotic conditions (Wang & Wondergem, 1992). Therefore, the present experiment was designed to test the hypothesis that L-alanine uptake-induced hyperpolarization of V_m provides an electromotive force for the redistribution of hepatocyte intracellular Cl^-, which may in turn contribute to cell volume regulation.

Materials and Methods

Adult, male mice (ICR strain) were purchased from Charles River Breeding Labs. (Charles River, MA) and were fasted 12–18 hr before experiments. The vivarium housing the mice met all standards of the American Association for Accreditation of Laboratory Animal Care. All inorganic chemicals were purchased from Fisher Scientific (Pittsburgh, PA), and organic chemicals were purchased from either Aldrich (Milwaukee, WI) or Sigma (St. Louis, MO).

Control and Experimental Solutions

Krebs physiological solution contained (in mM) 103 NaCl, 4.7 KCl, 2.56 $CaCl_2$, 1.3 $MgCl_2$, 25 $NaHCO_3$, 1.15 NaH_2PO_4, 2.8 glucose, 4.9 sodium pyruvate, 4.9 sodium glutamate, 2.7 sodium fumarate and it was equilibrated with 95% O_2–5% CO_2 (pH 7.46). L-alanine was added at 20 mM to experimental solutions. The osmolality of the control solution was adjusted to match that of the experimental solution by adding 20 mM sucrose. Osmolality of each experimental and control solution was measured with an automatic osmometer based on the principle of freezing point depression (Precision Systems, Natick, MA). Liver slice preparation, maintenance and temperature control were as described previously (Wang & Wondergem, 1993).

Fabrication of Open-Tip Microelectrodes and Ion-Selective Microelectrodes

Microelectrode preparation, use and criteria for valid impalements were as described previously (Wang & Wondergem, 1993). Liquid ion-exchanger [Corning 477913 for Cl^-, Corning 477317 for K^+ and TMA^+, and Na^+ exchanger prepared from Fluka's sodium ionophore I, ETH 227 as described previously (O'Doherty, Garcia-Diaz & Armstrong, 1979; Wondergem & Castillo, 1986)] was introduced into the back of the sialinized micropipette, filling the microelectrode tip with a column of exchanger ranging from 0.2–1.0 mm. The microelectrodes were backfilled with 0.5 M KCl for K-electrodes, Cl-electrodes and TMA-electrodes, and with 0.5 M NaCl for Na-electrodes. The open-tip (reference) barrel was filled with 1 M Na formate for K^+, TMA^+ and Cl^- measurements and with 0.5 M KCl for Na^+ measurements.

Calibration of Ion-Selective Microelectrodes

Ion-selective microelectrodes were calibrated in ionic solutions covering the physiologic or intracellular range. Ion activities in these calibration solutions were computed by modifications of the Debye-Hückel equation (Armstrong, Byrd & Hamang, 1973).

Cl^--selective microelectrodes are subject to interference by extracellular and intracellular anions, notably HCO_3^- and bile salts, respectively (Lyall, Croxton & Armstrong, 1987). For this reason, Cl^- microelectrodes were calibrated in 100, 50, 20, 10, 5 and 1 mM KCl solutions each containing fixed amounts of 25 mM $NaHCO_3$, 4.9 mM Na pyruvate, 4.9 mM Na glutamate, and 2.7 mM Na fumarate. The latter are the possible interfering anions of the extracellular Krebs medium. Cl-sensitive microelectrodes were calibrated according to methods described by Lyall, Croxton & Armstrong (1987). We described the details in a previous report (Wang & Wondergem, 1992).

K^+-selective microelectrodes were calibrated in 100, 50, 20, 10, 5 and 1 mM KCl solutions. Details of these methods have been described (Wang & Wondergem, 1991, 1993).

TMA^+-selective microelectrodes were calibrated in 50, 20, 10, 5 and 1 mM TMA chloride solutions plus 125 mM KCl in each solution. Details of these methods have been described (Khalbuss & Wondergem, 1991; Wang & Wondergem, 1993).

Na^+-selective microelectrodes were subject to interference from intracellular K^+, which is ~10-fold greater than Na^+ (Wondergem & Castillo, 1986). Consequently, a^i_{Na} was determined by interpolating intracellular voltages of the Na^+-selective microelectrodes onto calibration curves obtained from electrode voltages in varying NaCl solutions (2–100) mM, each containing a fixed amount of KCl at 132 mM. Activity of the latter (100 mM) approximates the mouse hepatocyte a^i_K measured by Wondergem and Castillo (1986). Slopes of the linear portions of calibration plots from three Na^+-selective microelectrodes ranged from 56 to 66 mM/10-fold change in Na^+ activity, with a mean ± SE of 62 ± 3 mM/10-fold change in Na^+ activity. Microelectrode selectivities for Na^+ over K^+, computed from these slopes by the fixed-interference method (Armstrong & Garcia-Diaz, 1980) ranged from 54.9 to 77.5 with a mean ± SE = 62.4 ± 7.5 (n = 3).

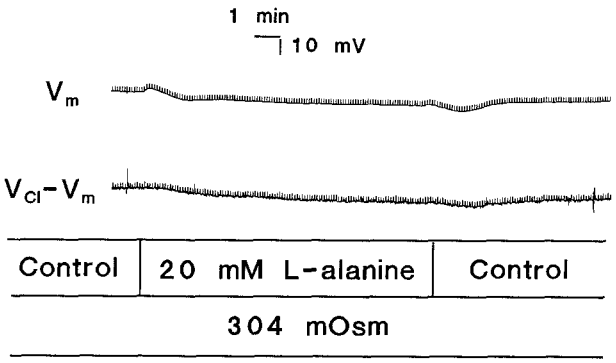

Fig. 1. Representative intracellular recordings of time-dependent changes in hepatocyte V_m and $V_{Cl} - V_m$ in response to 20 mM L-alanine. Increased electronegativity is downward for the V_m trace and upward for the $V_{Cl} - V_m$ trace. The downward deflection of $V_{Cl} - V_m$ indicates the decrease of a^i_{Cl}. Control values were recorded immediately prior to addition of L-alanine and experimental values were recorded when steady-state was achieved 7 min later.

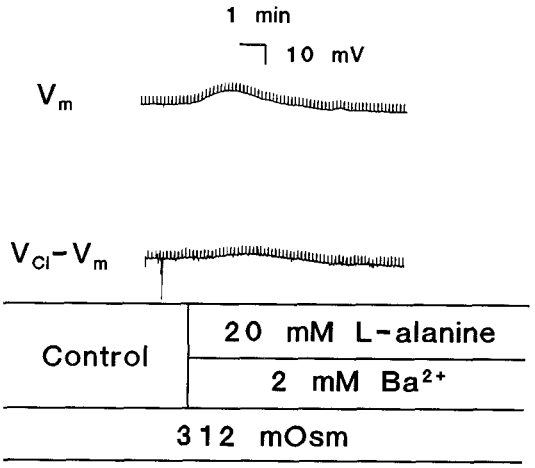

Fig. 2. Representative intracellular recordings show the coincident inhibitory effects of Ba^{2+} (2 mM) on the changes in hepatocyte V_m and $V_{Cl} - V_m$ induced by 20 mM L-alanine. Voltage polarities and recording times are indicated in the legend of Fig. 1.

Ion Activity and Cell Volume Measurements

When measuring the effect of L-alanine on V_m, intracellular ion activities, and cell water volume usually one impalement was made in each liver slice obtained consistently from a different animal. We have found that the response of the various parameters to L-alanine diminishes with repetitive measurements in the same liver slice. In each impalement, V_m and respective ion activities were measured continuously and steady-state values were recorded before (control) and following addition of 20 mM L-alanine (experimental). The experimental values were obtained one min after voltages had stabilized, which was 5 to 8 min after addition of L-alanine.

Statistical Analysis

Differences in paired means of control and after addition of L-alanine were determined by Student's t-test with significant differences at $P < 0.05$.

Results

The Effect of L-Alanine on Hepatocyte V_m

During L-alanine uptake, hepatocyte V_m depolarized transiently and then repolarized followed by sustained hyperpolarization. Representative traces of these changes in V_m induced by L-alanine are shown in Figs. 1 and 2. In 37 measurements, the V_m hyperpolarized by 18%, from -33 ± 1 to -38 ± 1 mV when liver slices were perfused with Krebs physiological solution containing 20 mM L-alanine for 5 to 8 min.

The Effect of L-Alanine on Hepatocyte a^i_{Na}

To test the assumption that hepatocyte intracellular Na^+ content increases during L-alanine uptake, a^i_{Na} was measured with double-barreled microelectrodes. In nine measurements, 20 mM L-alanine induced a 19% hyperpolarization of V_m, from -31 ± 1 to -36 ± 1 mV and a 30% increase in intracellular Na^+ activity, from 19 ± 2 to 25 ± 3 mM (Table 1). This result supports the concept that L-alanine intake in hepatocytes is accomplished by Na^+-dependent amino acid cotransport system.

The Effect of L-Alanine on Hepatocyte a^i_K

To test the general idea that L-alanine induces a net loss of intracellular K^+ in hepatocytes, a^i_K was also measured during L-alanine uptake. In 12 measurements, 20 mM L-alanine induced a 14% hyperpolarization of V_m, from -34 ± 1 to -38 ± 1 mV and a 5% decrease a^i_K from 83 ± 3 to 79 ± 4 mM (Table 1). The latter decrease, however, was insignificant.

The Effect of L-Alanine on Hepatocyte a^i_{Cl}

To test our hypothesis that hepatocyte intracellular Cl^- distributes passively with V_m during L-alanine uptake, both V_m and a^i_{Cl} were measured simultaneously with double-barreled microelectrodes. A representative trace for the change of V_m and $V_{Cl} - V_m$ induced by 20 mM L-alanine is shown in Fig. 1. The parallel changes of these two traces is readily seen. In seven measurements, 20 mM L-alanine induced a 24% hyperpolarization of V_m, from -36 ± 1 to

Table 1. The effects of L-alanine (20 mM) on the hepatocyte V_m, a^i_{Na}, a^i_K, a^i_{Cl}, a^i_T, and intracellular water volume

Experimental condition	N	V^c_m (−mV)	V^e_m (−mV)	V^e_m/V^c_m	V^e_m/V^c_m (−mV)	a^c_X (mM)	a^e_X (mM)	a^e_X/a^c_X	$a^c_X/a^e_X - 1$
Na^+	9	31 ± 1	36 ± 1[c]	1.19 ± 0.04	4 ± 1	19 ± 2	25 ± 3[b]	1.30 ± 0.07	
K^+	12	34 ± 1	38 ± 1[c]	1.14 ± 0.02	5 ± 1	83 ± 3	79 ± 4	0.95 ± 0.02	
Cl^-	7	36 ± 1	45 ± 2[c]	1.24 ± 0.03	9 ± 1	20 ± 2	12 ± 2[c]	0.62 ± 0.05	
TMA^+	9	31 ± 1	37 ± 2[c]	1.20 ± 0.03	6 ± 1	7 ± 1	6 ± 1[a]	0.9 ± 0.03	0.12 ± 0.03

V^c_m = hepatocyte resting transmembrane potential. V^e_m = transmembrane potential in experimental solution. a^i_{Na}, a^i_K, a^i_{Cl}, a^i_T represent intracellular Na^+, K^+, Cl^- and TMA^+ activities. N is the number of measurements. a^c_X =intracellular ion (X) activity in control condition. a^e_X = intracellular ion (X) activity in experimental condition. $a^c_X/a^e_X - 1$ represents the intracellular water volume change when intracellular TMA^+ is used as the marker. All values are averages ± SEM. [a]Differs from control, $P < 0.05$. [b]Differs from control, $P < 0.01$. [c]Differs from control, $P < 0.001$.

−45 ± 2 mV and a 38% decrease in intracellular Cl^- activity, from 20 ± 2 to 12 ± 2 mM (Table 1). Comparing the above V_m values with the Cl^- equilibrium potential (E_{Cl}) calculated from measured a^i_{Cl} values, 20 mM L-alanine increased E_{Cl} from −36 ± 2 to −49 ± 4 mV. These E_{Cl} values were in agreement with the V_m values, which was consistent with our hypothesis that Cl^- is in passive distribution with V_m.

The Effect of L-Alanine on Hepatocyte Intracellular Water Volume

The change in hepatocyte intracellular water volume was measured during L-alanine transport to determine whether corresponding changes in intracellular ionic activities resulted from cell swelling. In the nine measurements, 20 mM L-alanine caused a 20% hyperpolarization of the V_m, from −31 ± 1 to −37 ± 2 mV and a 10% decrease in a^i_{TMA}, which accounts for a 12% increase in hepatocyte intracellular water volume (Table 1).

The Effects of Barium on L-Alanine-Induced Changes of V_m and a^i_{Cl}

It has been proposed that the hyperpolarized V_m induced by L-alanine results from increased K^+ conductance, G_K, of the membrane (Kristensen & Folke, 1984). Thus, barium, a well-known K^+ channel blocker, should be able to block the hyperpolarized V_m, if the change of V_m is due to the increase in G_K. To test further the relationship between the change of V_m and that of a^i_{Cl}, V_m and a^i_{Cl} were measured simultaneously when 2 mM Ba^{2+} was present in the perfusate during L-alanine uptake. Compared with the normal condition, L-alanine-induced hyperpolarization of V_m was abolished completely by 2 mM barium ($n = 8$), and the decrease in a^i_{Cl} also was blocked entirely during L-alanine uptake. The V_m depolarized 2 mV more and did not undergo hyperpolarization in the presence of barium (Fig. 2, Table 2). In addition, the $V_{Cl} - V_m$ had a tendency to follow the depolarization of V_m during alanine uptake (Fig. 2) when the V_m repolarization-hyperpolarization was inhibited by Ba^{2+}. In this case, the L-alanine-induced increase in K^+ efflux was blocked, the time course for the depolarization of V_m was protracted, and the magnitude was larger. A corresponding upward deflection of $V_{Cl} - V_m$ trace indicated an increase of a^i_{Cl}, (Fig. 2). Finally, the V_m values in Table 2 were compared with the Cl^- equilibrium potential (E_{Cl}) calculated from measured a^i_{Cl} values. The E_{Cl} during 20 mM L-alanine uptake in the presence of 2 mM Ba^{2+} changed from −33 ± 2 to −32 ± 3 mV. These results strongly supported our hypothesis that Cl^- passively distributes with hepatocyte V_m during the change induced by L-alanine.

The Effect of Ouabain on L-Alanine-Induced Changes of a^i_K

Ouabain was used to determine the role of the Na^+-K^+ pump in the process of L-alanine uptake induced change in hepatocyte V_m. After perfusing liver slices for 20 min with Krebs physiological solution containing 1 mM ouabain, 20 mM L-alanine induced only a 7% hyperpolarization of V_m, from −31 ± 2 to −33 ± 3 mV, along with a 13% decrease in a^i_K from 61 ± 3 to 54 ± 5 mM. This percentage of decrease in a^i_K was much higher than that of the untreated liver slices (Table 3).

Discussion

Hyperpolarization of hepatocyte V_m during L-alanine uptake is thought to sustain the transmembrane electrochemical Na^+ gradient during electrogenic

Table 2. The effect of Ba^{2+} on the L-alanine-induced changes of V_m and a^i_{Cl} ($n = 8$)

Experimental condition	V^c_m (−mV)	V^e_m (−mV)	V^e_m/V^e_m	a^c_{Cl} (mM)	a^e_{Cl} (mM)	a^e_{Cl}/a^c_{Cl}
Untreated	36 ± 1	45 ± 2*	1.24 ± 0.03	20 ± 2	12 ± 2*	0.62 ± 0.05
Ba^{2+} (2 mM)	33 ± 1	32 ± 1	0.98 ± 0.05	22 ± 2	24 ± 3	1.07 ± 0.06

V^c_m = hepatocyte resting transmembrane potential. V^e_m = transmembrane potential in experimental solution. a^c_{Cl} = intracellular Cl^- activity in control condition. a^e_{Cl} = intracellular Cl^- activity in experimental condition. All values are averages ± SE *Differs from control, $P < 0.001$.

Table 3. The effect of ouabain (1 mM) on the L-alanine-induced changes of V_m and a^i_K ($n = 6$)

Experimental condition	V^c_m (−mV)	V^e_m (−mV)	V^e_m/V^c_m	a^c_K (mM)	a^e_K (mM)	a^e_K/a^c_K
Untreated	34 ± 1	38 ± 1[b]	1.14 ± 0.02	83 ± 3	79 ± 4	0.95 ± 0.02
Ouabain	31 ± 2	33 ± 3	1.07 ± 0.04	61 ± 3	54 ± 5[a]	0.87 ± 0.04

V^c_m = hepatocyte resting transmembrane potential. V^e_m = transmembrane potential in experimental solution. a^c_K = intracellular K^+ activity in control condition. a^e_K = intracellular K^+ activity in experimental condition. All values are averages ± SEM. [a]Differs from control, $P < 0.05$. [b]Differs from control, $P < 0.001$.

co-transport of Na^+ and amino acids (Kristensen, 1980). We propose that this hyperpolarization in addition may be important for cell volume regulation, because Cl^- distributes passively with V_m in mouse hepatocytes as shown here and previously (Wang & Wondergem, 1992). The decrease in a^i_{Cl} during membrane hyperpolarization with L-alanine uptake equals the increase in a^i_{Na}. Assuming an accompanying efflux of cations to preserve electroneutrality, the voltage-driven Cl^- may constitute sufficient transmembrane osmolyte exchange to compensate for part of the cell swelling due to hepatocyte accumulation of Na^+ and alanine.

Our finding that hepatocytes swell during alanine uptake agrees well with that of Kristensen and Folke (1984). They reported that isolated rat hepatocytes exposed to 10 mM L-alanine for 30 min swelled 1.15 times normal volume. We attribute slightly less swelling with 20 mM L-alanine to having not inhibited amino acid metabolism; whereas, the measurements of Kristensen and Folke (1984) were performed with added aminooxyacetate (AOA). We previously reported direct effects of AOA on hepatocyte V_m (Wondergem & Castillo, 1988). Notwithstanding this difference, our 12% increase in mouse hepatocyte intracellular water volume occurred within the same time course that a^i_{Cl} decreased by 38% during L-alanine uptake. We conclude, therefore, that this decrease in a^i_{Cl} cannot be explained by the 12% dilution from hepatocyte swelling. On the other hand, the L-alanine-induced decrease of a^i_{Cl} is blocked when the accompanying hyperpolarization of V_m is inhibited by Ba^{2+}, a K^+ channel blocker. The corresponding values for E_{Cl} equaled the V_m, which is consistent with passive distribution of Cl^- and that hepatocyte a^i_{Cl} depends on V_m.

We attribute the lack of response of $V_{Cl} - V_m$ during the initial, transient L-alanine-induced depolarization of V_m to delayed Cl^- efflux from the cell. This conclusion is supported by the delayed increase in a^i_{Cl} during L-alanine-induced depolarization that occurred when the repolarization-hyperpolarization of V_m was blocked by Ba^{2+} (Fig. 2). A similar lag in redistribution of hepatocyte Cl^- was observed by Graf et al. (1987), who reported voltage-dependent changes in a^i_{Cl} in isolated, rat hepatocyte couplets during current clamp step-changes in V_m. Our conclusion that hepatocyte a^i_{Cl} decreases in response to L-alanine-induced hyperpolarization is consistent with reports that membrane Cl^- permeability is high relative to other ions. In perfused rat liver, the calculated permeability coefficient for K^+ is $= 7.6 \times 10^{-8}$ cm/sec and the $P_{Cl} = 12.3 \times 10^{-8}$ cm/sec. The G_{Cl} is three times greater than G_K (Claret & Mazet, 1972). Others have reported that Cl^- crosses the liver cell membrane relatively rapidly (Lyall, Croxton & Armstrong, 1987; Moule, Bradford & McGivan, 1987). Thus, liver differs from other epithelia and cell types that actively transport Cl^- (Ussing, 1982) and whose membrane Cl^- permeability is a regulated variable, which is activated to initiate transmembrane water flux (Hudson & Schultz, 1988).

Fitz and Scharschmidt (1987*a*) reported that intracellular Cl^- activities are close to the level predicted for passive distribution under basal conditions and after hyperpolarization of the V_m by alanine (Fitz & Scharschmidt, 1987*a*). Conversely, Moule et al. (1987) assumed passive distribution of hepatocyte Cl^-, and they correlated changes in transmembrane Cl^- distribution with initial rates of L-alanine uptake to demonstrate that L-alanine transport resulted in hyperpolarization of V_m and stimulation of the Na^+-K^+ pump. However, conclusions from both studies were weakened by the absence of continuous intracellular recordings of V_m and a^i_{Cl} during the period of alanine uptake and by lack of corresponding measurement of change in cell water volume.

L-alanine uptake in hepatocytes is primarily through a Na^+-alanine cotransport system at a stoichiometry of 1 : 1 (Kristensen, 1986). Addition of alanine to the extracellular medium increases the influx of Na^+ and leads to an increase in the cellular Na^+ concentration (Kristensen, 1986). Meanwhile, a transient depolarization of the cell membrane occurs (Folke & Paloheimo, 1975). Our findings are in agreement, and we conclude that the initial depolarization in V_m during L-alanine uptake results from the influx of Na^+. Nonetheless, the subsequent hyperpolarization of V_m with loss of cell Cl^- and increase in cell Na^+ does not satisfy the law of electroneutrality within the intracellular compartment. We do not know at present what ions balance this apparent separation of charge.

Administration of 2 mM Ba^{2+} entirely abolished the L-alanine-induced hyperpolarization; however, it was blocked by only 50% when the Na^+-K^+ pump was inhibited. These findings show that the hyperpolarization of hepatocyte V_m after the transient depolarization results from the combined increase in K^+ permeability of the plasma membrane and activation of the Na^+-K^+ pump. We conclude that L-alanine-induced increase in K^+ conductance predominates among the factors that hyperpolarize the cell membrane. However, in addition to blocking the K^+ conductance, Ba^{2+} may inactivate the Na^+-K^+ pump indirectly by blocking the efflux of intracellular K^+. A corresponding rise in steady-state intracellular K^+ could inhibit activation of the Na^+-K^+ pump by competing for Na^+ binding on the cytoplasmic side (Skou, 1992).

L-alanine-induced increase in unidirectional K^+ efflux has been demonstrated by several groups (Kristensen, 1980; Bakker-Grunwald, 1983; Kristensen & Folke, 1984; Cohen & Lechene, 1990). Thus, it is possible that our time course (5–8 min) for K^+ measurement was not long enough to create a significant net loss of intracellular K^+ content. Kristensen reported that L-alanine added to isolated rat hepatocytes immediately decreases intracellular K^+ content, which reaches a new stable minimum level after 20 min (Kristensen, 1986). However, our result is consistent with the findings of Cohen & Lechene (1990), who showed that a 15 min exposure to 10 mM L-alanine did not alter the intracellular K^+ content in primary cultured rat hepatocytes. Nevertheless, they showed an increase in Na^+-K^+ pump rate, an increase in K^+ efflux, and an increase in the initial rate of K^+ loss after Na^+-K^+ pump inhibition by ouabain (Cohen & Lechene, 1990). Thus, they attributed the failure of L-alanine to reduce intracellular K^+ content to stimulation of the Na^+-K^+ pump.

Inhibition by ouabain of the Na^+-K^+ pump in liver slices causes loss of cell K^+ and equivalent Na^+ gain without cell swelling (McLaughlin, 1973; Macknight, Pilgrim & Robinson, 1974). This decreases the Na^+ transmembrane electrochemical gradient and consequently in the presence of ouabain less Na-alanine cotransport occurs (Moule et al., 1987). In this case our results underestimate the Na^+-K^+ pump activation during L-alanine uptake. In other words, L-alanine should produce an even bigger decrease of intracellular K^+ activity in the presence of ouabain than shown in Table 2, if the intracellular Na^+ activity were not elevated. Thus, our present study strongly suggests that L-alanine uptake stimulates both intracellular K^+ efflux and Na^+-K^+ pump activity simultaneously, so that the intracellular K^+ content remains constant in mouse hepatocytes. In addition to blocking the activity of the Na^+-K^+ pump, ouabain also blocks the alanine induced hyperpolarization of V_m. We have already shown that ouabain has no effect on hypotonic shock-induced hyperpolarization of V_m (Wang & Wondergem, 1993). This suggests that the mechanisms of hyperpolarization of V_m between alanine uptake and hypotonic shock are different. In the case of alanine uptake, both the increased K^+ conductance and the activated Na^+-K^+ pump activity contribute to the V_m hyperpolarization, while only the increased K^+ conductance is responsible for the V_m hyperpolarization during hypotonic stress. Since the main difference between these two conditions is the intracellular Na^+ content, it is very likely that an increase in intracellular Na^+ content is the major stimulation for efflux of K^+ and the Na^+-K^+ pump activity.

Adaptive responses to cell swelling from either hypotonic stress or nutrient transport comprise activation of membrane K^+ and Cl^- transport pathways in various cell types (Hoffman, 1978; Hudson & Schultz, 1988; Welling & O'Neil, 1990; MacLeod & Hamilton, 1991). These ion fluxes play an important role in regulating cell volume, and many cell sensors

and transduction mechanisms have been implicated (Chamberlin & Strange, 1989). For example, Colclasure and Parker (1992) have proposed recently that cytosolic protein concentration is the primary volume signal for swelling-induced K-Cl cotransport in dog red cells. Corresponding changes in phosphorylation potential constitute the effector response to the volume signal (Jennings & Schulz, 1991; MacLeod, Lembessis & Hamilton, 1992*a*), and changes in activity of intracellular divalent cations may play a regulatory role (Lauf, 1985; Wong & Chase, 1986). However, important recent findings suggest that different regulatory mechanisms effect adaptive increases in K-Cl cotransport, depending on whether cell swelling resulted from hypotonic stress or nutrient transport (MacLeod & Hamilton, 1991; MacLeod, Lembessis & Hamilton, 1992*a*,*b*).

In summary, we propose that cell volume control and regulation of cell K^+ during L-alanine transport into hepatocytes comprises the following mechanism: Hepatocyte L-alanine uptake through the Na^+-alanine cotransport systems increases hepatocyte Na^+ content and osmotic pressure. The rise in a^i_{Na} stimulates the Na^+-K^+ pump, while water moves into the cell due to the osmotic pressure gradient. Cell swelling by unknown mechanisms increases K^+ and Cl^- permeabilities of the plasma membrane. Both the activation of the Na^+-K^+ pump and the increase in K^+ conductance hyperpolarize V_m. The increase in V_m provides electromotive force for the efflux of Cl^-, which along with a cation to preserve electroneutrality of the intracellular compartment reduces cell swelling. In this model, Na^+ cotransported with L-alanine is pumped out of the cell, and Cl^- efflux effectively exchanges for L-alanine. The K^+ efflux is balanced by the K^+ pumped in and a^i_K does not decrease significantly.

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